

AWARD NUMBER: W81XWH-15-1-0079

TITLE: Lipid Neuroprotectants and Traumatic Glaucomatous Neurodegeneration

PRINCIPAL INVESTIGATOR: Dr. Sanjoy Bhattacharya

RECIPIENT: University of Miami School of Medicine
Coral Gables, FL 33146

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TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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14. ABSTRACT Glaucoma refers to a group of irreversible blinding diseases that steal sight slowly in increments without pain or obvious symptoms. Specific lipids naturally present in the clear fluid of the anterior chamber of the eye in healthy individuals but not in individuals suffering from glaucoma (endogenous lipids). The proposed research is to develop several such lipids as potential glaucoma therapeutics. The progressive loss of vision in glaucoma is often associated with elevated intraocular pressure (IOP). Lowering IOP remains the only proven strategy to halt glaucoma progression. Prostaglandins (not not other lipids) are currently the only known lipid that lowers IOP. About 25% of glaucoma patients including Veterans, are recalcitrant to available glaucoma medications; thus, new therapeutic molecules are needed to treat this group. The proposed research will further assess the efficacy of the new lipids to lower IOP using mouse and monkeys that can develop glaucoma naturally or by design. Trauma-induced glaucoma common among veterans are mostly non-responsive to conventional medication. The proposed research aims to provide a new mechanism-based therapy for such recalcitrant patients upon establishment of their efficacy in animal models.							
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1. INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Subject: To determine whether specific endogenous lipids lower intraocular pressure (IOP) in animal models and can be potential glaucoma medication.

Purpose: To evaluate specific endogenous lipids found in normal human eyes and determine their effect on lowering the IOP in the eyes of animal models (mouse, normotensive monkeys).

Scope of Research: To evaluate six lipids SBase1-4 and SM809, SM 839 on DBA/2J mice and cynomolgus monkeys for their IOP lowering effects. To test the hypothesis that they alter elastic TM, modulus and binding and functional assays with potential protein targets.

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

Endogenous lipids, Aqueous humor, Trabecular meshwork, Intraocular pressure, sphingolipids, primary cell culture, elastic modulus, protein targets.

3. ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major goal 1. Test the hypothesis that selected lipids uniquely present in the normal aqueous humor act as regulators of intraocular pressure. Methods, topical application of lipids, IOP measurements by miniified Goldman tonometer, Slit lamp examination of the eye (timeframe: months 4-18 Performed at University of Wisconsin, Wisconsin as a subaward; Prof. Paul Kaufman collaborator, cynomolgus monkeys will be used):

Percentage completion: About 60 percent complete. 2 lipids are remaining to be evaluated. These lipids have just been synthesized and will be evaluated soon.

Major goal 2. Test the hypothesis that the selected lipids lower intraocular pressure by reducing the resistance in the conventional outflow pathway. Methods, topical application of lipids, set pressure perfusion, fluorophotometry, episcleral pressure measurement, pathway analyses. (timeframe: months 4-36; at Case Western Reserve University, Cleveland as a subaward, Prof. Carol Toris, collaborator, DBA/2J mice will be used):

Percentage completion: About 40 percent complete. Additional lipids are being evaluated. The mouse in the Cleveland facility necessitated more time to acclimatize. The instrumental set ups were re-optimized and now the studies are ongoing.

Major goal 3. Test the hypothesis that selected endogenous lipids act as modulators of elastic modulus of trabecular meshwork tissue.

Percentage completion: These studies are 60% complete. Some newly synthesized lipids are being used for evaluation and determination of reproducibility.

Major goal 4. Test the hypothesis that the selected endogenous lipids alter trabecular meshwork cell behavior and exert their effects using membrane bound proteins.

Percentage completion: These studies are 60% complete. Some newly synthesized selected lipids are being used for evaluation and determination of reproducibility. The mass spectrometry data is being compared with functional data.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met.

Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major activities and 2) Specific Objectives (stated as goals). a) Intraocular pressure (IOP) has been measured in DBA/2J and normotensive monkeys (major goals 1 and 2). [These results are presented in Figure 1 below.](#) b) Attempts have been made to determine all parameters (including Aqueous outflow facility) and pathways (conventional/uveoscleral) in DBA/2J mice due to application of lipids (major goal 2). [These results are presented in Table 1 below.](#) c) Elastic modulus has been determined with human glaucomatous TM with and without these lipids and atomic force microscope (AFM). Further elastic modulus using high flow and low flow areas of glaucomatous TM was assessed with or without treatment with lipids (major goal 3). [These results are presented in Figure 2 below.](#) d) We have tested behavior of primary TM cells with or without lipids. We have also used various strategies including UV cross-linking to cross-link surface proteins with these lipids using TM cells. The lipid-cross-linked proteins were identified by mass spectrometry. These experiments are being repeated. Functional tests are underway for these proteins (major goal 4). [Some of these results are presented in Figure 3 below.](#) [We would like to emphasize that these are not final results yet. In some instances we are not entirely satisfied with some experimental results and would like to revisit a few experiments again. We will complete these additional experiments within the grant period. For some of these experiments we may utilize internal funds. Our own satisfaction with experimental results are important to us. We pay attention to minute details and sometimes even a small change causes some dissatisfaction to us, for example, the appearance of the cells under the microscope. Such situations are not entirely under our control nevertheless if we are not satisfied we want to repeat once again before using all data for final analysis.]

3) Significant results. We have found these lipids to lower the elastic modulus of glaucomatous TM and in particular, that of regions of low flow (pertain to major goal 3). We found that these lipids alter cell movements and interact with cell surface proteins. The lipids alter functions of selected cell surface proteins with which they interact (pertain to major goal 4). We have determined baseline outflow facility parameter in DBA/2J mice. Thus far the effect of lipids on the outflow facility could not be determined with certainty due to large intra-experimental error. We are re-evaluating these results. DBA/2J mouse presents great variability. In several independent experiments we have found significant IOP lowering (at Miami). However, experiments at Cleveland has not reproduced this results in a statistically significant manner (goals 2), Preliminary experiments have shown IOP lowering in monkeys for some lipids (3 lipids showed significant lowering on day 5th in monkeys).

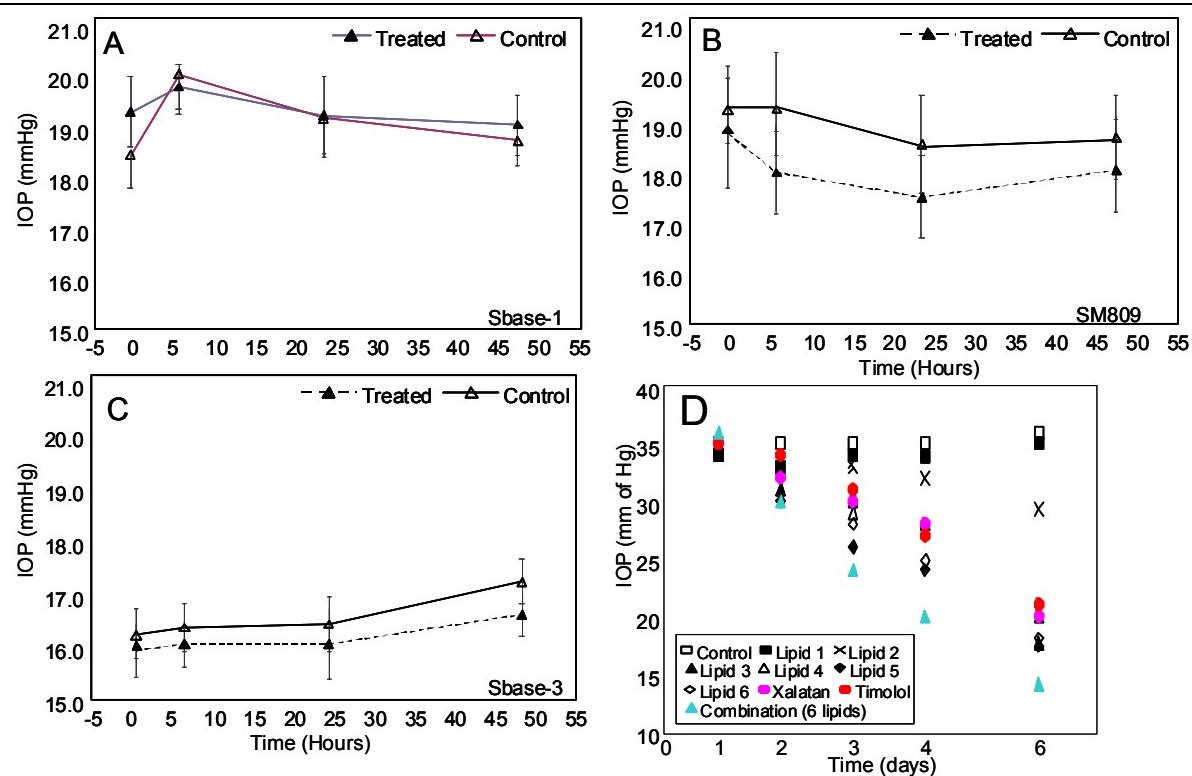


Figure 1. Representative intraocular pressure (IOP) after lipid treatment. A.-C. The cynomolgus monkeys were subjected to lipid treatment in a vehicle. The control (vehicle only) eye has and lipid treated eye readings are as indicated. The monkeys were also subjected to slit lamp examination by two independent observers to determine any adverse effect due to lipid treatment (no adverse effect was found). D. The effect of lipids on lowering in DBA/2J mice ($n=25-28$) for six lipids and a comparison with Xalatan and Timolol.

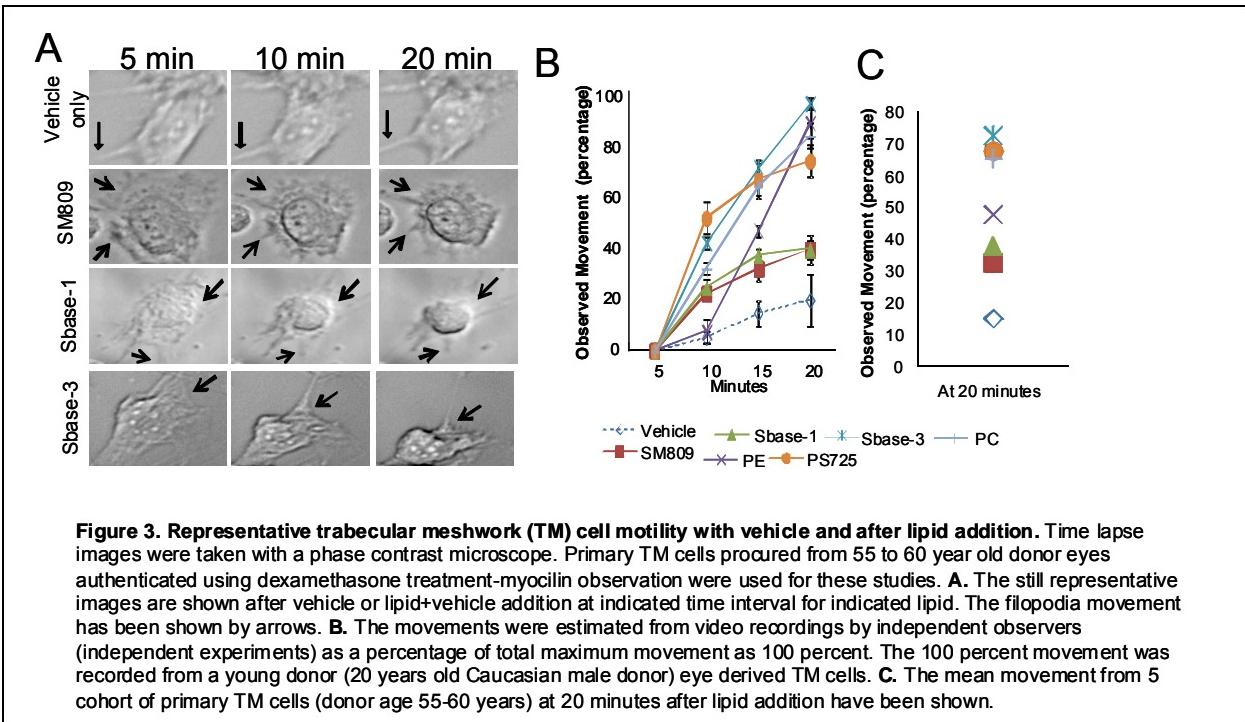
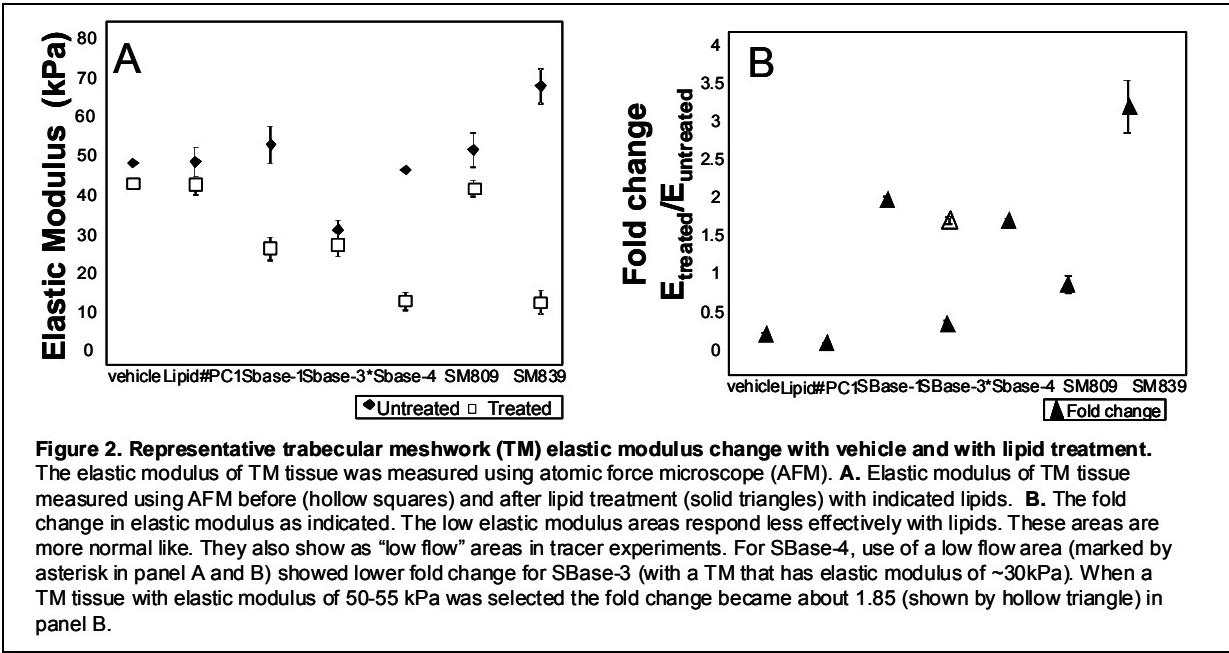


Table 1. Outflow parameter investigation in DBA/2J mice

Parameters	Control	+Sbase-1	+Sbase-3
Aqueous flow, $\mu\text{L}/\text{min}$ (Fa)	0.052 ± 0.031	0.09 ± 0.003	0.08 ± 0.05
Outflow facility, $\mu\text{L}/\text{min}/\text{mmHg}$ (C)	0.011 ± 0.002	0.09 ± 0.01	0.03 ± 0.01

All mice were around 8 months of age, Mean \pm standard deviation from n=8 mice have been presented

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. “Training” activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. “Professional development” activities result in increased knowledge or skill in one’s area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Training. A graduate student Genea Edwards learned: a) use of mass spectrometry for lipid analyses, b) measurement of elastic modulus, c) cell behavior assays including video imaging, d) UV cross-linking and mass spectrometry as well as various functional assays. She also underwent through animal research training.

A laboratory technician (Research Associate III) Ms. Mabel Algeciras learned lipid analyses using two different types of mass spectrometers and cross-linking and identification of lipid-bound protein targets.

Medical students and undergraduate students learned a lot of bioinformatic analyses, database building, cell culture and mass spectrometric methods due to this project.

Sruthi Sampathkumar, a postdoctoral student at Case Western Reserve University (Lab of Prof. Carol Toris) was trained in aqueous humor dynamic studies while working on this grant.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

The results were disseminated to undergraduate students, medical students in the form of seminars and posters. Two high school students during summer (as part of their HHMI research program) learned hands-on about lipid analyses. A University of Miami wide announced lecture was delivered for dissemination.

Dr. Bhattacharya also presented an invited seminar at Case Western Reserve University (<http://case.edu/med/ophthalmology/Seminars/2016SeminarSeries.html>) on February 10th, 2016. The manuscripts pertaining to these studies are yet to be written.

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state “Nothing to Report.”

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Major goal 1). We will continue evaluation with rest of the candidate lipids as planned and complete it.

Major goal 2). We will continue evaluation with rest of the candidate lipids as planned and complete these studies. We will continue to measure IOP lowering in DBA/2J mice in both Cleveland and Miami.

Major goals 3) and 4) Thus far these results are encouraging and insightful. We will continue these studies as planned and complete them.

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The greatest impact generated from these studies is putting importance of all classes of lipids for glaucoma in the "map". In several small glaucoma meetings classes of lipids are now being discussed in contrast to only prostaglandins.

The lipids in the study are demonstrating to play a role in cell behavior and trabecular meshwork tissue behavior. At the completion of these when these details are better understood, it is poised to exert a wide impact in this area.

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

As a consequence of these studies and dissemination in local and other small meetings, we were approached by people studying cancer or metastasis as well as people studying gastrointestinal aspects of metabolism. The colleagues in these fields utilizing our recently developed expertise published some initial findings, namely, "Phospholipid makeup of the breast adipose tissue is impacted by obesity and mammary cancer in the mouse (PMID: 25450252)" and "Stool phospholipid signature is altered by diet and tumor (PMID: 25469718)". Thus our studies are generating an impact beyond the immediate field of trabecular meshwork and glaucoma. Our cell behavior studies are poised to have an impact in other fields as well.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

We have a prior patent application on 11 lipids. We are negotiating with some private enterprises for potential sponsorship. If the new and non-overlapping lipids in this research cross any utility threshold some mechanisms will be in place for technology transfer. We will also potentially consider formation of a start-up if some results are superbly promising in multiple model systems.

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

In the academic world the results from these projects is likely to provide additional tools (lipids) to tweak cell and tissue behavior. The project will vastly improve our knowledge of lipids of different classes and the effect of select lipids in cells/tissue.

- 5. CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:

Nothing to report.

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

There have been insignificant changes in some approaches, for example, we are more focused on determining effect of lipids on outflow facility parameter amongst aqueous humor dynamic parameters. However, we will evaluate all other parameters. Thus only insignificant and incremental changes in approaches will take place. The research work is progressing as planned. There may not be desired or spectacular outcome but that is the nature of research. Again the work is progressing exactly as planned.

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

There may be a little delay in aqueous humor dynamic (AHD) studies. In Cleveland some changes in DBA/2J mice corneal properties were noted from one season to the other (winter versus summer). We expect to complete these studies within the total duration of the grant period. Thus far the studies on different AHD parameters have resulted in inconclusive inferences due to large intra-experimental variability. This may be intrinsic to DBA/2J mice with respect to AHD parameters. There is no prior published study on AHD parameters on this string.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable. Nothing to report.

Significant changes in use or care of vertebrate animals.

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report

- 6. PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume; year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Major publications. Nothing to report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Several book chapters in Methods in Molecular Biology (Springer) is pending.

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Dr. Bhattacharya also presented an invited seminar at Case Western Reserve University (<http://case.edu/med/ophthalmology/Seminars/2016SeminarSeries.html>) on February 10th, 2016 .

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Development of selected lipids. Bioinformatic methods for quantification of lipids using different approaches.

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

Identify any other reportable outcomes that were developed under this project.

Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Several lipid databases (all internal at this point). Their dissemination will require careful considerations. Selected lipids and knowledge of the activities (cellular effects) of these lipids.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change.”

At Miami

Name: Sanjoy K. Bhattacharya
Project Role PI
Nearest person month worked 3.6
Contribution to Project: Overall study designing, coordinating the experiments, reviewing data, generating report, considering presentations, disseminations, potential patent and commercialization opportunities, manuscript preparation, interpretation of data.
Consultation with experts in different areas.
Funding Support The effort captured here is funded by DOD grant. The PI has other including NIH funding.

Name: Noel Ziebarth
Project Role Co-investigator
Nearest person month worked 0.6
Contribution to Project: Assisted in designing atomic force microscopy experiments, interpretation of AFM data and preparation of summary of the results.
Funding Support The effort captured here is funded by DOD grant. Dr. Ziebarth has other funding support.

Name: Mabel Algeciras
Project Role Research Associate III (Lab Technician)
Nearest person month worked 9.6
Contribution to Project: Perform experiments, maintain animals and make sure regulatory needs are properly followed. Ordering chemicals and supplies and lab maintenance.
Funding support:

Name: Genea Edwards
Project Role: Graduate student

Nearest Person month worked 8
Contribution to Project: Perform IOP, elastic modulus and other cell behavioral and cross-linking mass spectrometry studies.
Funding support: NIH T32 training grant. Ms. Edwards is supported by a training grants for her PhD thesis work, which encompasses proposed project work. Thus she is supported by a training grant and working on this research under supervision of Dr. Bhattacharya.

At Wisconsin

Name Prof. Paul L. Kaufman
Project Role co-investigator
Nearest person month worked 0.12
Contribution to Project: Oversees the overall scientific, technical and administrative conduct of this subcontract. Has interacted with Dr. Bhattacharya and UW research staff regarding protocol design and implementation.
Funding support: Efforts for this project were supported by DOD grant. His laboratory is otherwise supported by several funding agencies for other projects.

Name	Julie Kiland
Project Role	Technician
Nearest person month worked	1
Contribution to Project:	Coordinated subcontract studies, drafted experimental protocols in consultation with Drs. Kaufman and Bhattacharya, coordinated animal acquisition, performed IOP experiments, as well as compiled and formatted data for review.
Funding support:	Efforts for this specific project was supported by DOD funding.

Name:	Elizabeth Hennes-Beean
Project Role:	Research Specialist
Nearest person month worked	1
Contribution to Project:	Assisted with all IOP experiments, coordinated animal treatments, and post-procedure monitoring, ordered and maintained supplies specific to this study
Funding Support:	Efforts for this specific project was supported by DOD funding.

At Cleveland

Name:	Carol Toris
Project Role:	co-investigator
Nearest person month worked	0.12
Contribution to project:	Assisted designing and interpreting aqueous humor dynamic (AHD) studies.
Funding support:	For this project funded by DOD grant. She has other funds to support her laboratory.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name: 1. University of Wisconsin (Prof. Paul Kaufman); Case Western Reserve University (Prof. Carol Toris).

Location of Organization: Wisconsin and Ohio

Partner's contribution to the project (identify one or more)

- ;
- Facilities;
- Collaboration (partner's staff work with project staff on the project);

Nothing to report except to state participation of Drs. Kaufman and Toris laboratories as mentioned above and detailed in other sections.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Lipid neuroprotectants and traumatic glaucomatous neurodegeneration

W81XWH-15-1-0079 (MR130303)



PI: Sanjoy Bhattacharya

Org: University of Miami

Award Amount: \$999,998

Study/Product Aim(s)

- Specific Aim 1. Determination of lipid mediated IOP lowering in cynomolgus monkeys.
- Specific Aim 2. Determination of pathway (conventional or uveoscleral) utilized by the lipids to increase aqueous outflow .
- Specific Aim 3. Determine whether elastic modulus of TM is modulated by lipids.
- Specific Aim 4. Determination of cell behavior and identification of protein targets by UV cross-linking and mass spectrometry.

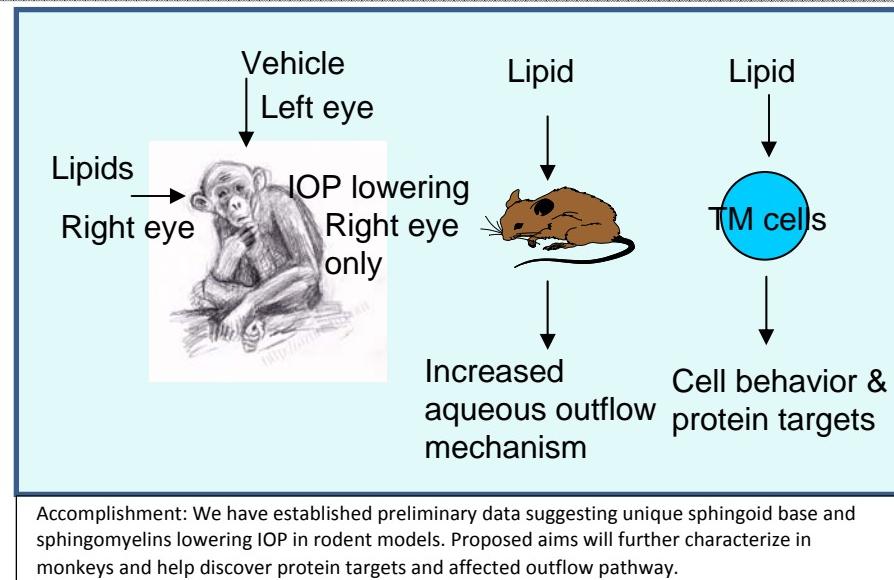
Approach

We will utilize cynomolgus monkeys to determine effect of endogenous sphingoid base and sphingomyelins on lowering IOP. We will use DBA/2J mice to investigate their effect to increase outflow by conventional and/or uveoscleral pathway. We will utilize tissues , primary TM cells to investigate the effect of these lipids on elastic modulus, cell behavior. We will utilize fluorescent lipids, UV cross-linking & mass spectrometry to investigate the protein targets of lipids.

Timeline and Cost

Activities	CY	15	16	17	18
Aim 1 (IOP evaluation)					
Aim 2 (Outflow pathway analysis)					
Aim 3 (Elastic modulus estimation)					
Aim 4 (Cell behavior analyses & protein identification)					
Estimated Budget (\$K)	\$250	\$333	\$333	\$83	

Updated: (4/29/2016)



Goals/Milestones

CY15 Goal – All model system, reagents and plan

- Determining study design & strategies. Gathering models and reagents.

CY16 Goals – IOP lowering by lipids and outflow pathway determination

- Assessment of IOP lowering in cynomolgus monkeys
- Increased outflow

CY17 Goal – Outflow pathway and elastic modulus determination

- Outflow pathway identification & elastic modulus determination.

CY18 Goal – Cell behavior & protein target identification

- Establish the lipid induced changes in cell behavior and cross-linking lipid analogs to determine their protein targets

Comments/Challenges/Issues/Concerns

- If timelines change, comment here. Not applicable (N/A)

Budget Expenditure to Date Not applicable.

Projected Expenditure: \$253K (year1)

Actual Expenditure: \$234K (year 1; till April 29, 2016)